

Studies in Brownian Movement. I.—On the Brownian Movement of the Spores of Bacteria.

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I. Brownian movement, the persistent and irregular motion of small particles suspended in a fluid, has been shown by the work of Wiener,* Jevons,† Gouy,‡ and others to be independent of such conditions as vibration, intensity of illumination and convection currents; it occurs equally at the surface of the earth and in deep cellars or tunnels. Its activity, in fact, seems to depend only on the size of the particles, and upon the temperature and viscosity of the fluid in which they are suspended. These facts led Wiener, and subsequently several others, to attribute the movements to the molecular motion of the fluid, of which the particles thus act as indicators.

In 1905 Einstein§ embodied this theory in a definite form: he showed that the total displacement of a particle in any given horizontal direction in a given time is proportional to the square root of that time, and is given by the equations

$$D = X^2/2t = RT/6\pi r\eta N,$$

in which D = coefficient of diffusion, X^2 = mean square of displacement in the direction of an arbitrary horizontal axis in time t , T = absolute temperature, r = radius of a particle, η = viscosity of suspending fluid, R = the gas constant 83.2×10^6 , and N = Avogadro's constant, the number of molecules in a gramme-molecule of a gas. Thus measurements either of D or of X and t , together with a knowledge of T , r , and η , allow N to be calculated, and its value compared with that obtained by other methods.

The correctness of this theory has been strikingly confirmed by the series of experiments carried out by Perrin and his fellow workers.|| They have used both measurements of the coefficient of diffusion and of the displacement of particles in a given time in their verifications, and have also

* 'Pogg. Ann.,' 1863, vol. 118, p. 79.

† 'Proc. Manch. Soc.,' 1869, vol. 9, p. 78.

‡ 'Journ. de Phys.,' 1888, vol. 7, p. 561.

§ 'Ann. der Physik,' 1905, vol. 17, p. 549.

|| 'Comptes Rendus,' 1908, vol. 146, p. 967; 1908, vol. 147, p. 475; 1909, vol. 149, pp. 477 and 549; 1911, vol. 152, pp. 1380 and 1569; 'Ann. de Chim. et de Phys.,' 1909, Ser. 8, vol. 18, p. 5.

examined rotatory Brownian movement, for which Einstein* also supplied the theory. Perrin's first experiments were made upon the statistical equilibrium of suspensions. Small particles of equal size suspended in a fluid of density less than their own tend to settle to the bottom of the fluid; this tendency is opposed by their Brownian movement, which, if it were not for gravity, would distribute them evenly throughout the fluid. The net effect is thus a distribution exactly similar to that of the air molecules in an isothermal atmosphere; the number of particles in a given horizontal layer is statistically constant as soon as a kinetic equilibrium has been established, and the ratio of the numbers in any two strata at a vertical distance h apart is given by the equation, for suspensions,

$$\log \frac{n_0}{n} = \frac{NV}{RT} (\Delta - \delta) gh,$$

where n_0 is the mean number per unit volume measured at the lower level, n that at the upper level, Δ and δ the densities of the material of the particles and of the fluid respectively, and V the volume of each particle. The numbers n_0 and n of this equation and X in that of Einstein's theory were determined by microscopic observations of suspensions enclosed in a glass cell. The suspensions were bounded both at bottom and top by the glass of the microscope slide and cover-slip respectively; the observations were thus made on what we call below "enclosed drops."

The mean values of $N/10^{22}$ determined were:—

Equilibrium of suspensions	68.2
Brownian movement of translation ...	68.8
Brownian movement of rotation	65
Diffusion of suspensions	69

The conditions were varied in the series of experiments to the following extent:—

Mass and volume of particles, in the ratio of 1 to 70,000 and 1 to 90,000 respectively.

Viscosity of liquid, 1 to 330.

Difference, $\Delta - \delta$, between densities of particles and liquid, 1 to 30.

Temperature, from -9° C. to 58° C.

Nature of suspending liquid: water; sugar and urea solutions; glycerine, pure and diluted with water.

Nature of suspended particles: gamboge and mastic.†

* 'Ann. der Physik,' 1906, vol. 19, p. 371.

† J. Perrin, 'Les Atomes,' Lib. Felix Alcan, 1913, 3rd Edit., p. 188.

These variations would appear to be amply sufficient for a satisfactory test with the exception of the last. The mere agreement of the results obtained with two such similar substances as mastic and gamboge, both resins, is not in itself of much value. The close accordance of the value of N obtained in these various experiments with the values derived from entirely different methods is however strongly in favour of Einstein's theory, as a complete, and not merely an approximate, quantitative description of Brownian movement. The opinion of other observers, that the movements are independent of the nature of the suspended particles, though not supported by exact measurements, must yet be accorded some value. Nevertheless it seems desirable to examine the behaviour of particles quite different from resins, and particularly to investigate any apparent anomalies in Brownian movement.

II. In a previous paper* by one of us and Dr. S. B. Walsh, it was recorded that the spores of certain bacteria in suspension in various liquids did not show Brownian movement. The spores examined, those of *Bacillus anthracis*, *B. subtilis* and others, are approximately ellipsoidal, of dimensions about $1.2\mu \times 1\mu \times 1\mu$. The observations were made upon hanging-drop preparations.

The spores forming the suspensions at first exhibited Brownian movement, but after a short time a very large proportion of them became entirely stationary. Small particles of other substances, such as carbon, and non-motile cocci, such as *Staphylococcus aureus*, of about the same size, in exactly similar preparations, continued in Brownian movement indefinitely.

The immobility of the spores was shown to be independent of temperature and occurred in water, in saline solutions, in formol solutions, and in dilutions of glycerine of marked differences of density and viscosity. The immobility was also found to take place whether the spores were living or had been previously killed by heat.

The only way in which it was found possible to inhibit the immobility of spores in hanging-drop preparations was by treating them with antiformin (solution of sodium hydrate and sodium hypochlorite), a strongly alkaline liquid with great powers of attacking organic matter. After this treatment spores showed persistent Brownian movement comparable with that of ordinary particles. Acids were without effect; the characteristic immobility appeared as usual in hanging-drop preparations of spores which had been treated with 50-per-cent. hydrochloric acid for 48 hours.

III. The further observations to be recorded show that in a hanging-drop preparation the immobile spores form a layer, one spore thick, at the air-water interface, movement in the plane of the interface as well as in the

* 'Brit. Med. Journ.,' November 9, 1912, p. 1305.

vertical direction being completely arrested. In the course of time these immobile spores congregate, still at the interface and still in a layer one spore thick, at the lowest, most dependent portion of the drop. Provided the spore has reached the interface, the attainment of immobility is independent of the direction of its long axis relatively to the surface film. The comparatively few spores which do not settle into close approximation to the air-water interface continue to show Brownian movement.

Hanging-drop preparations were also made with particles, of roughly equal size, of carbon, mastic, sulphur, barium sulphate, *Staphylococcus aureus*, etc. All these particles remained in free Brownian movement right down to the bottom of the drop, *i.e.*, they were not affected by the air-water interface.

The formation of the immobile layer was expedited by heat, either by making a hanging drop with nearly boiling water, or by heating after the drop was made; this is probably because of the lessened viscosity, and to a smaller extent the diminished density of the hot water, which permit the spores to gravitate more quickly to the air-water interface.

Spores are bodies of considerable density (about 1.3 gm. per cubic centimetre) but this cannot explain their immobility, several of the other substances used being much denser, *e.g.*, sulphur and barium sulphate. To definitely show that their weight played no part in the immobility at the bottom of the drop, an inverted drop was examined—that is, a drop placed on the microscope slide itself, protected from draughts and evaporation by a cover-slip resting on a vaseline ring without touching the drop. The film of immobile spores was again found at the air-water interface, this time at the top of the drop. This observation is again alluded to below.

Nor is the immobility a result of agglutination; although in densely populated suspensions the spores of the immobile layer are close together, especially about the centre of the surface, they show not the slightest signs of agglutination, either in two or three dimensions. Sparse suspensions were made in which only a few spores appeared in the field of view, and these spores showed the characteristic immobility as soon as they reached the air-water interface, without any attempt at aggregation.

IV. It appeared then that the peculiar behaviour of spores might be due to a surface tension effect, that the spores were in some way able to become part of the surface of the hanging drop, exchanging their kinetic energy of Brownian movement for the potential energy of the surface film.

The cover-slip of a hanging-drop preparation of anthrax spores in which immobility had occurred was lifted, placed on a vaseline ring on a flat

slide, and pressed down until the drop came into contact with the glass of the slide. The suspension, now contained between glass surfaces both above and below, was at once examined, and it was found that all the spores exhibited Brownian movement.

Spores in such enclosed-drop preparations are still found chiefly in the lowest portions of the drop, their numbers decreasing rapidly from below upwards, as in Perrin's suspensions of resin particles. But, although the populated region is thus shallow, by reason of the high density of spores as compared with the fluid, it was in no sense a plane, or a surface such as that seen in the immobile layer of a hanging-drop preparation.

Thus, in a suspension of anthrax spores bounded above and below by glass, the distribution usual in suspensions is found—the number of spores in a given horizontal stratum is statistically constant. But if the medium below is air, then the spores close to the air surface become immobile, and a further supply of spores comes down, to be in turn brought to rest. The process continues until practically all the spores have collected into an immobile layer, one spore thick, at the bottom of the drop. Even after a considerable lapse of time, however, a few spores remain in suspension, and consequently continue to show Brownian movement. This does not indicate that they are of a different nature from the immobile spores: that a few spores should remain free for very long periods is to be expected from theoretical considerations. The complete mathematical solution of the state of the suspension, after the lapse of any given time from the moment of formation of the drop, is a difficult matter, depending as it does upon the initial distribution of spores and upon the rate of immobilisation as compared with the rate of settling under gravity; but the problem may be simplified by making the assumptions: (1) that the suspension is initially in the state of statistical equilibrium represented by Perrin's formula in §I above, and (2) that the rate of immobilisation is so slow that it does not disturb this form of distribution of the spores remaining in suspension at any instant. These two assumptions practically amount to the one, that the suspension must have time to settle into the state described in the equation before any large proportion of the spores has been brought to rest. This appears to be very approximately true: at all times throughout the process of immobilisation, as long as any considerable number of spores remain in the body of the liquid, the population in the field of view is observed to grow less and less dense as the microscope tube is raised.

Let $n_0 dx$ be the number of spores at any instant in a column of unit cross-section and depth dx , immediately above the air-water interface, $n dx$ those in a similar layer at a height x above, then $n = n_0 e^{-kx}$, where k is a constant.

Hence if s is the total number of spores in the whole vertical unit column,

$$s = \int_0^{\infty} n dx = \frac{n_0}{k}.$$

The rate of immobilisation at the foot of the column at the instant considered is clearly given by the expression cn_0 , where c is a constant. Hence $ds/dt = -cn_0 = -cks$, which therefore gives $s = Se^{-ckt}$, where S is the total number of spores in the column initially. Thus the number of spores remaining in suspension only becomes zero after an infinite lapse of time: the observation that some few spores still remain in the body of the fluid and retain their Brownian movement for a long period is thus accounted for.

If an immobile layer contains so many spores as to leave no free air-water interface whatever, the upper side of the spore layer acts just as glass would do in an enclosed-drop preparation, with the result that Brownian movement persists indefinitely among the spores compelled to remain in suspension.

V. The immobility of a spore is not produced until it reaches a distance from the air-water interface too small to be measured. If the microscope is focussed on the lowest part of a hanging drop while the process of settling is going on, spores can be observed in all stages from free Brownian movement to complete immobility. On limiting the depth of field in focus by increasing the diameter of the illuminating beam it can always be demonstrated that the most freely moving particles are the highest, and the quite stationary ones the lowest, but the whole depth through which the transition occurs is certainly a small fraction of 1μ . This would of course be expected if the phenomenon of immobility is a result of surface tension, since Lord Rayleigh and others have shown that the effective thickness of surface films is of the order of a few micromillimetres.

When the upper surface of the drop was the one exposed to air the immobile layer was found to exist from the first at the air-water interface. The greater number of spores gravitated to the bottom of the drop and formed there a population exactly like that of an enclosed-drop preparation, Brownian movement continuing indefinitely. The upper layer of spores, *i.e.* those at the air-water interface, did not fall through the drop but remained immobile. These spores were not, of course, further recruited from the main body of the drop, and were evidently those which happened to be at the interface when the drop was prepared. The immobile spores immediately acquired Brownian movement when the cover-slip was pressed down into contact with the drop, producing an enclosed drop.

In repeating this observation it happened that at a certain part of the margin of the enclosed drop the preparation extended under the cover-slip

for a short distance so as to prolong the attachment of the suspending fluid to the cover-slip as compared with the usual more or less abrupt limit of the drop. In this way there was produced a kind of mixed drop, the extension under the cover-slip being of the nature of a shallow hanging drop, the rest of the preparation an enclosed drop. The usual layer of motionless spores appeared at the bottom of the shallow hanging drop; that these spores were not dried on to the cover-glass was shown by the fact that a few free spores were seen moving above them. It was observed that the layer of immobile spores followed the downward curve of the meniscus bounding the main enclosed drop; immediately within that drop, just above the glass of the slide, the spores of the lower layer exhibited the usual Brownian movement of enclosed drops. It was further possible to see the air-water interface edgewise, half way down the meniscus at the boundary of the enclosed drop; only those spores which appeared to be in actual contact with the interface were motionless, those which were within the drop retained their movements up to a distance of less than 1μ from the meniscus. This directly confirms the statement above that the distance from the interface at which spores lose their Brownian movement is exceedingly small.

To remove any doubt as to whether the spores at the air-water interface might be in some sense dry, enclosed drops, in which every spore was in Brownian movement, were separated into two drops by lifting the cover-slip. It was found that the spores which were left at the air-water interfaces of both the drops thus formed were quite immobile, however quickly the preparations were examined.

VI. The immobile spores seem to lie in a veritable surface skin, for even violent stirring of a drop by a platinum loop fails to restore any considerable number to the body of the liquid. A hanging drop, which was stirred while under observation, showed that the skin of spores was dragged about upon the surface of the drop, but not broken up to any extent. Again, if the microscope stage be tilted through a considerable angle while a hanging-drop preparation is being viewed, the immobile spores merely move slightly with the drop as a whole, while the spores exhibiting Brownian movement in the body of the drop drift rapidly above them across the field.

In some enclosed-drop preparations small air-bubbles occurred; large numbers of spores adhered to their surfaces, and in all cases these spores were absolutely motionless. They were in the fluid, closely attached to the surface of the bubble. Spores in free Brownian movement were noticed at distances less than 1μ from the bubbles. When by cautious pressure upon the cover-glass a bubble was made to move about, the spores were

seen to remain in position upon its surface, rolling with it. Very violent rolling resulted in tearing off the surface film in strips, and the curious appearance was presented of flat tails or ribbons, about $30\ \mu$ long and $5\ \mu$ broad, composed of immobile spores. These ribbons trailed out from the bubble into the liquid, leaving bare patches of corresponding shape upon the surface of the bubble. The ribbons were presumably held together by minute traces of air, which, being gradually absorbed by the liquid, set free the spores, which broke away one by one to exhibit Brownian movement.

VII. Xylene, paraffin oil, and aniline oil were added to different enclosed-drop preparations of anthrax spores suspended in water. At the interfaces between water and the added liquid, in each case, spores were observed which were quite motionless, whilst those in the water continued their Brownian movement as usual. No spores could be demonstrated within the xylene or paraffin, but the aniline contained a number which were in motion. In certain preparations small drops of xylene were noticed in the water, and their surfaces were studded with immobile spores, just as in the case of the air-bubbles described above. Greasing the slide caused a similar immobility at the bottom of an enclosed drop.

Control preparations of *Staphylococcus aureus* showed none of the peculiarities which we have described—free Brownian movement persisted indefinitely up to the surfaces of air-bubbles, xylene, etc., and in no case was a film of immobile cocci produced.

VIII. The treatment with antiformin alluded to in §II was carefully repeated. Anthrax spores were suspended in 50-per-cent. antiformin and subjected to a temperature of 37°C . for 20 minutes. The suspension was then centrifuged, and the supernatant liquid discarded; the deposit was washed with water and again centrifuged. A suspension of the final deposit was made with water and examined as a hanging drop. The spores were considerably and irregularly reduced in size, and their surfaces appeared to have undergone a corrosive action; the high refractivity usual in spores was now much less marked. In these hanging drops there appeared no trace of the usual immobility, either immediately or after the lapse of days. Occasionally an immobile spore could be found at the air-water interface, but such spores were always large and highly refractile, appearing to have escaped corrosion by the antiformin. The bulk of the particles, as stated, remained in free Brownian movement down to the bottom of the drop, the smallest being, of course, the most active.

A number of untreated spores were added to such a hanging drop, with the result that the immobile layer developed as usual, and was made up

of large highly refractile particles, evidently the added spores; the small corroded spores retained their Brownian movement. The continued movement after treatment with antiformin is thus due to an alteration of the surface of the spore, and not to any peculiarity of the liquid resulting from imperfect washing of the centrifuged deposits.

IX. *Summary*.—(1) Bacterial spores examined in hanging-drop and in resting-drop preparations cease their Brownian movement and exhibit immobility at the air-water interface, an immobility involving loss of all Brownian movement even in the plane of the interface. The only subsequent movement is closer aggregation in the interface, due to gravity, as a result of the slope of the surface of the drop.

(2) The immobile spores appear to lie in a veritable skin at the surface of the drop.

(3) Resumption of Brownian movement by immobile spores at the air-water interface can only be induced by (*a*) destruction of the interface by conversion of the hanging or resting drop into an enclosed drop, or partially and with considerable difficulty by violent stirring of the spores at the interface, so as to incorporate them into the body of the drop; (*b*) alteration of the surface of the spores by the action of corrosives.

(4) The phenomenon of immobility is also noted when spores are found at the interface between water and certain "oils," *e.g.*, paraffin, aniline, xylene, and grease.

X. *Discussion*.—The phenomenon of immobility of spores, under the conditions referred to above, indicates a peculiarity in the nature of their surfaces, distinguishing them from other particles of similar size. Since the immobility occurs only at the interfaces between the suspending liquid and other fluids, the effect can be reasonably attributed to surface tension. As the presence of spores at the interface constitutes a final state of stable equilibrium it follows that the attainment of this state is accompanied by a decrease of the potential energy of the system; one way of interpreting this is to suppose that the surface tension is lowered for that portion of the suspending liquid in the immediate vicinity of the immobile spore. On this supposition the phenomenon is closely allied to the increase of concentration in the surface layers of certain solutions, *viz.*, those in which the increase of concentration is accompanied by decrease of surface tension. In such solutions the surface potential energy is reduced by some of the dissolved substance leaving the interior to augment the concentration of the surface film. This excessive surface concentration has been fully discussed by Willard

Gibbs,* Sir J. J. Thomson,† and others, and has been demonstrated in the froths of solutions of amyl alcohol, sodium oleate, etc., by Miss Benson,‡ Zawidzki,§ and Milner.|| Further, substances have been shown actually to separate out in a more or less solid form from saponin and other solutions and suspensions by Ramsden¶ and Shorter.**

It must be noticed that, while the fact that spores remain at the interface, and are unable to break away from it, may be thus accounted for, no satisfactory explanation is in this way given of the absence of Brownian movement in the plane of the interface; such motion would not require the doing of work against surface forces, and should therefore be possible. The immobility may be due to an abnormally high viscosity in surface films. The existence of such a viscosity has been asserted by many writers: Plateau, Marangoni, and more recently Lord Rayleigh†† and Shorter (*loc. cit.*) have dealt with the subject. The viscosity may be probably due in some way to a surface contamination; in the present case it is difficult to see how a "clean" surface, in Lord Rayleigh's sense of the term, could be obtained; indeed, the spores themselves may be the "contamination."

Two factors, then, may be regarded as entering into the phenomenon of the immobility of bacterial spores at fluid interfaces: (1) a diminution of surface energy, due to some peculiarity of the spores, this causing them, as soon as they are brought in any way within the range of action of the surface forces, to be drawn into close approximation to the interface; and (2) the abnormal viscosity of surface layers of liquids, which destroys the Brownian movement of the spores. Particles other than spores, not producing a lowering of surface energy, are not drawn into such close contact with the interface, and so do not experience the surface viscosity.

The viscosity concerned must be far greater than that of glycerine, for these layers of immobile spores show not the slightest trace of movement under the highest magnifications, while the Brownian movement of spores in enclosed drops of pure glycerine is readily observable. This, however, is probably not an objection to the hypothesis here put forward. Surface viscosity produces a remarkable effect upon the motions of bodies as

* 'Scientific Papers,' 1906, vol. 1, Paper III, "On the Equilibrium of Heterogeneous Substances."

† 'Applics. of Dyn. to Phys. and Chem.,' 1888, pp. 190 and 251.

‡ 'Journ. Phys. Chem.,' 1903, vol. 7, p. 532.

§ 'Zeit. f. phys. Chem.,' 1900, vol. 35, p. 77; 1903, vol. 42, p. 612.

|| 'Phil. Mag.,' 1907, vol. 13, p. 96.

¶ 'Roy. Soc. Proc.,' 1903, vol. 72, p. 156.

** 'Phil. Mag.,' 1909, vol. 17, p. 560.

†† 'Roy. Soc. Proc.,' 1890, vol. 47, p. 281; 1890, vol. 48, p. 127.

relatively vast as magnetic needles and rings of wire, whose inertia is very great as compared with that of spores, and the proportion of the surface exposed to the action of a surface viscosity is also much greater for a spore of dimensions only about 1μ than for a needle or wire.

There is at present no evidence of an electrical factor in the phenomenon, but further work on this point is in progress.

The Transmission of Cathode Rays through Matter.

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The experiments of this paper are the outcome of an attempt to discover whether cathode rays are selectively absorbed when their speed is sufficient to excite the radiation characteristic of the material they traverse.

The general laws of absorption of cathode rays were elucidated many years ago by Lenard,* and the law which bears his name is expressed by the relation

$$I = I_0 e^{-\lambda x},$$

where I_0 is the "intensity" of a beam of cathode rays incident on an absorbing sheet of thickness x , and I is the "intensity" of the emergent rays. The intensity, as measured by Lenard, was indicated by the brightness of a phosphorescent screen struck by the rays. In the above formula λ is the absorption coefficient. For a definite initial speed of ray λ/ρ is constant, ρ being the density of the absorbing material, while for any material Lenard found that λ varied inversely as the fourth power of the speed of the rays.

In the present experiments the number of the cathode rays incident on and emergent from various metallic sheets is studied.

The general disposition of apparatus is shown in fig. 1, which is not drawn to scale (ebonite insulation is shaded in the diagram).

T is a narrow brass tube passing through an ebonite plug tightly fitting the small brass cylinder C. The top of C consists of the metal leaf under examination, the leaf and the material of C being in close contact. D is another brass cylinder of slightly larger diameter insulated from C by an

* Lenard, 'Wied. Ann.,' vol. 56, p. 255 (1895).